(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: 0 380 719
A1

EUROPEAN PATENT APPLICATION

(21) Application number: 89101543.0

(51) Int. Cl.5: C08B 37/10, A61K 31/725

(22) Date of filing: 30.01.89

(43) Date of publication of application:
08.08.90 Bulletin 90/32

(84) Designated Contracting States:
DE ES FR GB IT NL SE

(71) Applicant: AJORCA S.A.
Paraná 378, 2 piso
AR-1017 Buenos Aires(AR)

(72) Inventor: Diaz, Victor Bautista
Av. R.S. Pena 852, 6 piso "A"
1035 Buenos Aires(AR)
Inventor: Dománico, Ricardo Hugo
Av. R.S. Pena 852, 6 piso "A"
1035 Buenos Aires(AR)
Inventor: Fussi, Fernando
Via Bagutti 22
CH-6900 Lugano(CH)

(74) Representative: Abitz, Walter, Dr.-Ing. et al
Abitz, Morf, Gritschneder, Freiherr von
Wittgenstein Postfach 86 01 09
D-8000 München 86(DE)

(54) Modified heparins and obtention process.

(57) The present invention relates with modified heparins having antithrombotic activity.

The invention also relates with a process of preparing such modified heparins starting from water insoluble heparin ammonium quaternary complexes.

Finally, the invention relates with pharmaceutical compositions containing the above modified heparins.

EP 0 380 719 A1

Modified heparins and obtention process

This invention refers to modified heparins having antithrombotic activity by oral administration, a method to obtain said heparins and pharmaceutical compositions containing them.

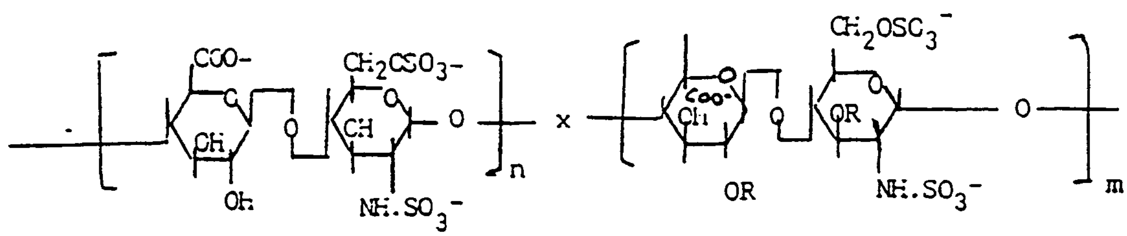
Heparin is one of the best known natural substances used in therapy due to its unreplaceable anticlotting and antithrombotic activity.

Heparin is a complex heteropolysaccharide made by repeating disaccharide units. Each unit is made by a uronyl residue bound to glucosamine-O- and N-sulfate.

Uronic acids in the sequence are alternatively: D-glucuronic, L-iduronic, L-iduronyl-2-sulfate.

Glucosamine is sulfated in positions corresponding to the amino and 6-hydroxy groups. In some sequences also a peculiar glucosamine trisulfate is found, having an extra hydroxy group in position 3. This glucosamine trisulfate, although representing only a small fraction of the total glucosamine in the heparin molecule, is basically significant for the anticlotting activity.

On this basis, the natural heparin could be describ ed under the following formula:



where: R = H or $-SO_3$- m/n:2:1 m + n = about 20(average)

This formula shows that the only hydroxy group that is always free in the heparin molecule is the one in position 3 of the uronic acid.

The many different biological and pharmacological activities of heparin are the consequence of intricate and partially not well understood biomolecular mechanisms. Actually, we know that the anticlotting activity of heparin is related to its specific link with a protease pro-inhibitor known as antithrombine III (At-III). The specific site in the heparin for the bond formation with At-III is a pentasaccharide sequence having glucosamine tri-sulfate in its centre.

On the other hand, the heparin anticlotting activity is not strictly necessary and proportional ot its antithrombotic activity.

On the contrary, in many instances it is desirable to reduce the anticlotting activity whenever this activity can be identified with a hemorrhagic effect. In fact, some modified heparins having low anticlotting activity show an optimal antithrombotic effect. These preparations are considered as a great improvement on normal heparins, as they act with a therapeutical action of paramount importance in the prophylaxis of thrombosis jointly with a lower risk of hemorrhage.

It is now accepted that the antithrombotic effect can be roughly evaluated in vitro through the assay of the specific factor X activated inhibition (anti-Xa activity).

Unfortunately, heparin is active only when administered by parenteral route and is not orally absorbed.

The low or null biodisponibility of the orally administered heparin hampers its use in long term therapies and prevents patients in "thrombogenic state" from keeping constant levels of anti-Xa activity.

Heparin is not orally absorbed essentially for 3 reasons:

1) It is a high molecular weight polymer (12000-15000 D) and there are no enzymatic mechanisms in the digestive tract or in the bloodstream able to split heparin molecule.

2) It is an extremely hydrophylic substance (partition coefficient in n-octanol/water less than 0.01).

3) It is a highly ionizable compound.

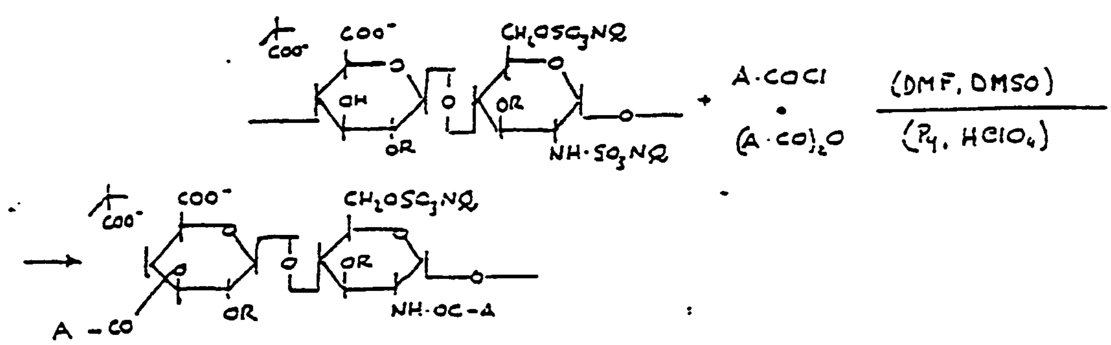
The heparin controlled depolymerization can be obtained through various methods described in the literature: nitrous acid treatment, beta-elimination, peroxydolysis, atomic oxygen action, molecular sieve fractionation. Therefore, at present, it is quite simple to obtain heparin fractions having a mean molecular weight ($\overline{NW}$) lower than the commercial heparin, showing high anti-Xa and low anticlotting activity (LMW Heparins), but depolymerization, per se, is not sufficient to attain an oral heparin. In order to obtain by semi-synthesis new molecules of heparin derivates with high anti-Xa activity as well as the possibility of oral absorption, we have settled an original process which allows us, just in one step, both to substitute the sulfate group in the amino nitrogen of the heparin and to esterify specifically the hydroxy group in position

3 of the uronyl residue with the same acyl groups. Undoubtedly, this double substitution occurs if the hydroxy group has not been previously etherified or esterified.

Our process to obtain heparins intended for oral administration, as claimed in the present invention, starts with heparins complexed with ammonium quaternary basis (NQ^{+}). The complexes must be insoluble in water and therefore easily isolable. They are well known in the literature, as for instance, complexes prepared with cetyl trimethyl ammonium or cetyl pyridinium halides. These complexes, although insoluble in water, can be split in saline solutions and they are soluble or can be solubilized in hydrate form in some organic solvents such as dimethylformamide (DMF), dimethylsulfoxide (DMSO) or acetic anhydride.

In the reaction of the present invention (see scheme 1), made with organic chlorides or anhydric in presence of an acyl carrier (pyridine or perchloride acid), the complexing agent $-NQ^{+}$ acts as a protector, an esterification orienter and permits to run the reaction in a homogeneous phase.

Scheme 1



where: R = H or SO_3NQ

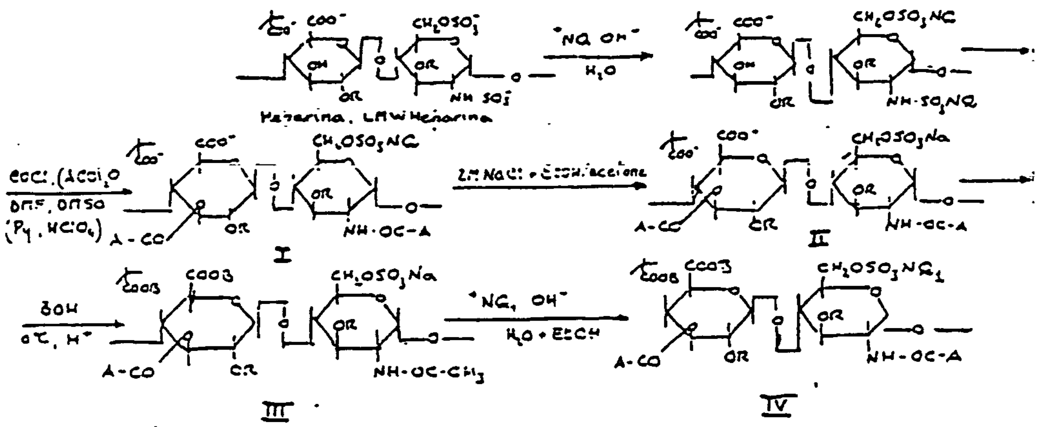
A = alkyl, aryl or alkyl-aryl groups.

In a further step, -NQ is easily removed by splitting the product of reaction in a saline solution (as for instance, 2 M NaCl) and precipitating the compound by addition of a water-miscible solvent (ethanol, methanol, acetone), where NQ salts are soluble and therefore can be removed.

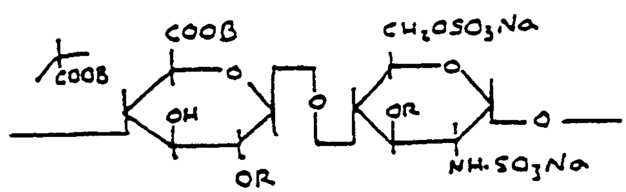
Compounds like shown in scheme 2, are obtained in this way. These compounds are less hydrophylic and less ionized than the heparin and show high anti-Xa activity. More hydrophylic and more ionized compounds are obtainable if the uronic carboxy group is esterified before or after the reaction (see scheme 2, III) and if the residual sulfate groups are complexed, after the completion of the above-mentioned reaction, with quaternary ammonium bases (NQ1), able to form water-soluble complexes in water, as for instance choline betaine, carnitine, tetraethyl or tetrabutyl ammonium hydroxides (see scheme 2, IV).

As a whole, the entire process described in scheme 2, permits to obtain compounds II, III, IV, in high yield. All these compounds are endowed with anti-Xa activity and with less hydrophylia and ionicity than the starting heparin.

Scheme 2



If an esterification on the carboxy group has been made before the acylation step, intermediate compounds with formula V, and their related complexes (VI), can be isolated:



V

If the hydroxy group in position 3 of the uronyl residue has been already blocked by esterification or etherification, acylation will occur only in the amino group.

This pattern is useful in case it is desirable to obtain compounds having different substituents in the hydroxy group 3 and in the amino group.

The following examples, not limiting for the claims, describe better the procedure leading to products II, III, anc IV of scheme 2.

Example 1

a) 20 g of a complex LMW Heparin-CTA (LMW Heparin = 5500 D, USP potency = 71 u/mg, anti-Xa = 90 u/mg; CTA = cethyl trimethylammonium) are suspended in 100 ml DMF and 10 ml pyridine are added. Heat to 60° C and add, by dropping and stirring, 8 ml of benzoyl chloride. The reaction is completed in 20 minutes. Add 1000 ml of 2% NaHCO, solution in water. Collect the precipitate (I) and dissolve in 100 ml of a 117 g/l NaCl solution. Precipitate with 300 ml of ethanol/acetone 1 : 1 mixture Re-dissolve the precipitate in 30 ml of NaCl solution as above and reprecipitate with 100 ml ethanol/acetone. Collect the precipitate, dehydrate with acetone and vacuum dry. Yield: 10 g of II, showing positive reaction to hydroxylamine/Fe^{III}, USP potency = 27 u/mg, anti-Xa = 80 u/mg., APTT = 23 u/mg.

b) 10 g of II are suspended in 100 ml butanol at 5° C and added with 0,5 ml of conc. HCl. Leave during 72 hours at 5° C by stirring. Filter and repeatedly wash with acetone. Vacuum dry. Yield: 9 g of III showing very positive reaction with hydroxylamine/Fe^{III}, USP potency: 23, anti-Xa = 85, and APTT = 19.

c) 9 g of III are dissolved in 50 ml of DI-water, add 1,50 g of choline chloride and precipitate the complex IV by additon of three volumes of a mixture 1:1 ethanol/acetone. Repeat this last step. Vacuum dry. Yield: 9 g of IV with USP potency = 25, anti-Xa = 90 and APTT = 17.

Example 2

a) 20 g of V-CP complex (obtained by butilation of the uronic carboxy group in the heparin and subsequent isolation of the complex with cetylpyridinium chloride CPC) are suspended in 100 ml DMF and added with 10 ml pyridine and 5 ml DI-water. Heat to 50 °C and add by dropping and stirring 10 ml acetic anhydride. The reaction is completed in thirty minutes. Proceed as in Example 1a). Yield: 10 g of III, showing a strong positive reaction with hydroxylamine/Fe^{III}, USP potency = 25 and anti-Xa activity = 90.

b) 9 g of III are dissolved in 50 ml of water. Proceed as in Example 1c). Yield: 9 g of IV with USP potency = 22, anti-Xa = 92.

Example 3

a) 20 g of Heparamine-3-butyl ether-CTA complex (obtained by N=desulfation of heparin, follwed by etherification of uronic hydroxy group in position 3 and isolation of the complex obtained with cethyl trimethyl ammonium halide), are suspended in 100 ml of acetic anhydride and added with 0,5 ml of concentrated perchloric acid. Heat during 30 minutes by stirring at 30 °C; add 800 ml of DI-water, collect the precipitate and dissolve it in 100 ml of a 177 g/l NaCl solution. Proceed as in Example 1a. Yield: 10 g of II, showing positive reaction with hydroxylamine/Fe^{III}, USP potency = 10 and anti-Xa = 75.

b) 10 g of II are suspended as in Example 1b). Yield: 9g of III, having a strong reaction with hydroxylamine/Fe^{III}, USP potency = 12 and anti=Xa = 84.

c) 9 g of III are processed as in Example 1c). Yield: 9 g of IV. USP potency = 12 and anti-Xa = 90.

Table 1 hereinbelow enclosed summarizes comparative data for some of the compounds described in the examples, compared with the starting materials.

TABLE I

PRODUCT (Ref.: Scheme 2 and Examples 1 to 3)	$\overline{MW}$ (Calc.)	SCHEMATIC CHEMICAL FORMULA	USP POTENCY/mg	ANTI-Xa/mg	APTT/mg.
USP HEPARIN	12.500	COONa; OH; $NHSO_3Na$	150	150	150
LMW HEPARIN	5.500	COONa; OH; $NHSO_3Na$	71	90	60
II (Ex 1)	6.536	COONa; OBz; NHBz	27	80	23
III (Ex 1)	7.024	COOBut; OBz; NHBz	23	85	19
IV (Ex 1)	8.040	COOBut; OBz; NHBz; x Chol.	25	90	17
V	5.988	COOBut; OBz; $NHSO_3Na$	64	95	53
III (Ex 2)	5.969	COOBut; OH; NHAc	25	90	18
IV (Ex 2)	6.049	COOBut; OAc; NHAc; x Chol.	22	92	15
II (Ex 3)	4.796	COONa; OAc; NHAc	10	75	8
III (Ex 3)	5.095	COOBut; OBut; NHAc	12	84	11
IV (Ex 3)	5.808	COOBut; OBut; NHAc; x Chol.	12	90	10

Abbreviations: Ac = Acetyl ; Bz = Benzoyl ; But = butyl chol. = Choline.

Claims

1. Modified heparins having antithrombotic activity for oral administration, characterized by the presence of acyl esters in the amino group of glucosamine and in the hydroxy group 3 in the uronyl residue.

2. Heparins according to claim 1, in which the uronic carboxy group is free or esterified.

3. Heparins according to claims 1 or 2, in which the sulfate groups are complexed with quaternary

ammonium basis having C_1-C_4 substituents, able to form water-soluble complexes.

4. Heparins according to claims 1 to 3, characterized by having the following properties: octanol-water partition coefficient higher than the starting heparin, conductivity lower than the starting heparin, anti-Xa equal or higher than the starting heparin, total anticlotting activity equal or lower than the starting heparin.

5. A method to obtain the aforesaid heparins according to claim 1, in which the acylation is performed on the amino and hydroxy groups in homogeneous phase, in one step, starting from water insoluble heparin ammonium quaternary complexes, operating in non-aqueous solvents capable to dissolve the complexes, by reaction with organic chlorides or anhydrides in presence of an acyl-carrier catalyst, at a temperature between room temperature and 80° C.

6. Pharmaceutical compositions containing heparins according to claim 1, including an adequate excipient and the aforesaid heparins.

European Patent Office

EUROPEAN SEARCH REPORT

Application Number

EP 89 10 1543

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
X	EP-A-0 256 880 (MIAMI UNIVERSITY) * Whole document *	1-6	C 08 B 37/10 A 61 K 31/725
Y	CARBOHYDRATE RESEARCH, vol. 59, 1977, pages 285-288, Elsevier Scientific Publishing Co., Amsterdam, NL; S. HIRANO et al.: "Some N-acyl derivatives of N-desulphated heparin" * Page 286, paragraph 2 *	1-6	
Y	US-A-4 331 697 (KUDO et al.) * Column 4, line 20 - column 5, line 37 *	1-6	
Y	CARBOHYDRATE RESEARCH, vol. 46, 1976, pages 87-95, Elsevier Scientific Publishing Co., Amsterdam, NL; Y. INOUE et al.: "Selective N-desulfation of heparin with dimethyl sulfoxide containing water or methanol" * Whole document *	1-6	**TECHNICAL FIELDS SEARCHED (Int. Cl.5)**
Y	AGR. BIOL. CHEM., vol. 40, no. 12, 1976, pages 2501-2502; S. HIRANO et al.: "N-acylation of N-desulfated heparin" * Whole document *	1-6	C 08 B A 61 K
A	US-A-4 510 135 (TENG)		

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	05-10-1989	LENSEN H.W.M.

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document